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Divergent composition and turnover of soil organic nitrogen along a climate gradient in arid and semiarid grasslands

Jiao Feng[a,b], Benjamin L. Turner[c], Kai Wei[a], Jihui Tian[a], Zhenhua Chen[a], Xiaotao Lü[a], Chao Wang[a], Lijun Chen[a,*]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China
[c] Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Panama

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ABSTRACT

Greater aridity predicted with climate change in drylands worldwide will affect soil nitrogen (N) cycling and the associated ecosystem functions. Despite > 90% of soil N occurring in organic forms, the pathways of soil organic N (SON) turnover remain largely unknown in drylands, where biological activity is typically limited by water availability. Here we examined patterns of SON fractions and soil N-hydrolyzing enzyme activities across a 3700 km aridity gradient in arid and semiarid grasslands of northern China. We found that both the concentrations of all SON fractions and the proportion of more stable SON increased with increasing aridity index (AI, defined as mean annual precipitation/potential evapotranspiration). The largest SON fraction was hydrolysable NH_4^+ in arid sites, but amino acid-N in semiarid sites. The activities of enzymes that hydrolyze relatively stable SON polymers (protease, peptidase, and *N*-acetyl-β-glucosaminidase) were negligible in arid sites (AI < 0.2), but increased significantly as AI increased in semiarid sites (AI > 0.2). Structural equation modeling indicated that the direct effect of microbial biomass on soil amidase was insignificant in arid sites, indicating that microbial SON turnover via enzymes is relatively weak. In semiarid sites, however, microbial biomass exerted significant direct positive effects on all soil N-hydrolyzing enzymes, suggesting strong microbial regulation of SON turnover via enzymatic mineralization. Altogether, our findings provide empirical evidence for divergent patterns of storage and turnover of SON between arid and semiarid grasslands.

1. Introduction

Drylands account for 47.2% of the global land area, but are threatened by desertification as a result of increasing aridity (Austin et al., 2004; Feng and Fu, 2013). As a major constraint for plant growth and primary productivity, soil nitrogen (N) availability is considered a key control on soil quality and ecosystem functioning in drylands (Aranibar et al., 2004; Austin et al., 2004; Wang et al., 2014). Given that > 90% of N in surface soil occurs in organic forms (Stevenson, 1982), the release of plant available N from soil organic N (SON) is the primary control on soil N availability and soil N cycling (Nannipieri and Eldor, 2009). However, dryland soils are inherently of low fertility, contain little soil organic matter (SOM), and have slow decomposition rates (Gallardo and Schlesinger, 1992; Zhou and Zhang, 2014), so the microbial regulation of SON turnover in drylands has received relatively little attention (Sinsabaugh et al., 2015). Increasing aridity in drylands depletes SOM and nutrients (Austin et al., 2004; Delgado-Baquerizo et al., 2013), indicating that SON turnover can respond to changes in climatic factors in drylands. This, in turn, could affect the responses of soil N biogeochemical cycles and related ecosystem functions to future changes in global climate.

The release of plant available N from SON is driven by a sequence of different enzymes, among which the commonly measured are those targeting proteins (protease), polypeptides (peptidase), chitins (chitinase) and amides (amidase) (Kandeler et al., 2011; Nannipieri and Eldor, 2009). In drylands, soil N-hydrolyzing enzymes are sensitive to variations in climatic factors (Ladwig et al., 2015; Sinsabaugh et al., 2008). For instance, increasing precipitation can directly increase soil

Abbreviations: N, nitrogen; SON, soil organic nitrogen; SOM, soil organic matter; MAP, mean annual precipitation; MAT, mean annual temperature; PET, mean annual potential evapotranspiration; AI, aridity index; ANPP, above-ground net primary production; SOC, soil organic carbon; TN, total nitrogen; NAG, *N*-acetyl-β-D-glucosaminidase; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MUB, 4-methylumbelliferone; AMC, 7-amino-4-methylcoumarin; OLS, ordinary least squares; RDA, redundancy analysis; SEM, structural equation modeling; MB, microbial biomass; SONC, first component of soil organic nitrogen fractions

* Corresponding author at: Institute of Applied Ecology, Chinese Academy of Sciences, P. O. Box 417, Shenyang 110016, China.
E-mail address: ljchen@iae.ac.cn (L. Chen).

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N-hydrolyzing enzyme activities by increasing the solubility of organic N substrates (Ladwig et al., 2015; Schjønning et al., 2003). In addition, the productivity and community structure of plant and microbial biomass vary with increasing aridity in drylands (Maestre et al., 2012; Wang et al., 2015), which can also interact to affect soil N-hydrolyzing enzyme activities (Geisseler et al., 2010; Kandeler et al., 2011). In soils containing an array of organic substrates, the degradation of different compounds requires microbial allocation of energy and resources to many distinct enzymes (Allison et al., 2011; Lehmann et al., 2008). However, it is difficult to make direct comparisons of enzyme activities in response to climatic changes among different studies in drylands, due to the measurements of various types of enzymes as well as the spatial variations in edaphic conditions (Evans et al., 2011; Ladwig et al., 2015; Sinsabaugh et al., 2015).

The mineralization of SON via enzymes is tightly related to the concentration and quality of SON compounds (Geisseler et al., 2010; Nannipieri and Eldor, 2009). Numerous studies have reported gradual accumulation of SON as precipitation increases in drylands (Austin and Vitousek, 1998; Wang et al., 2014), but few have examined the chemical nature of the SON. The acid hydrolysis method is effective in fractionating SON into more detailed chemical and functional pools (Bremner, 1965; Stevenson, 1982). Different SON fractions have various characteristics and decomposition potentials (Lü et al., 2013). In arid soils, SON may be less protected through re-adsorption with the soil matrices because of the low contents of both SOM and fine particles such as silt and clay (Evans et al., 2011; Parton et al., 1987; Wang et al., 2014; Wang et al., 2016), leading to lower proportions of complex SON forms (Sowden, 1977; Stevenson, 1982). Given the low concentration of SON in drylands (Post et al., 1985; Wang et al., 2014), even slight changes in chemical characteristics and availabilities of SON forms could transform a site from an N source to a sink or vice versa, exerting influences on soil N mineralization, N storage as well as soil fertility and quality (Nannipieri and Eldor, 2009).

The arid and semiarid grasslands of northern China are fragile regions threatened by global climate change, due to the extreme water limitation as a consequence of the low precipitation coupled with high evapotranspiration (Feng et al., 2016; Wang et al., 2014). Therefore, cross-site investigations covering large-scale aridity gradients in this region can offer insights into the possible implications of future climate change for SON turnover and ecosystem N cycling. Here, we sampled soils from 52 sites along a 3700 km aridity gradient in arid and semiarid grasslands of northern China. We determined the patterns of various N-hydrolyzing enzyme activities and chemical fractions of SON across the aridity gradient. In addition, we analyzed the direct and indirect effects of climatic and edaphic factors on SON fractions and various N-hydrolyzing enzyme activities using structural equation modeling. The objectives of this study were to investigate (1) how N-hydrolyzing enzymes are allocated to affect soil N cycling across the aridity gradient, and (2) how climatic and edaphic factors interact to affect SON stored in different forms and the subsequent SON turnover via enzymes across the aridity gradient.

2. Materials and methods

2.1. Site, climate and soil sampling

The study was performed along a 3700 km west-east grassland transect across the Xinjiang, Gansu Province and Inner Mongolia (Fig. 1). The climate along the transect is temperate continental, characterized by high precipitation and temperature during the growing season (between May and August). The transect mainly follows a climate gradient, with distinct variations in the mean annual precipitation (MAP) and mean annual temperature (MAT). Climate data i.e., MAT, MAP and mean annual potential evapotranspiration (PET) for each sampling site were obtained from the WorldClim database (Hijmans et al., 2005) using ArcGIS version 9.3 (Esri, Redlands, CA). Along the transect, MAP ranges from 34 mm to 436 mm, and is strongly negatively ($R^2 = 0.889$, $P < 0.001$) correlated with MAT (between −3 °C and 10 °C) (Feng et al., 2016). The aridity index (AI, calculated as the ratio of MAP to PET) includes the effect of both MAP and MAT (United Nations Environment Programme, 1992) and is a more accurate metric of water availability. The AI increases from 0.03 to 0.57 along the transect from west to east, indicating increasing humidity. The ecosystem type changes gradually from arid grassland to semiarid grassland as AI increases, with an AI boundary of 0.20 (United Nations Environment Programme, 1992). The dominant grassland types are desert shrub, desert steppe, typical steppe and meadow steppe, sequentially as the AI increases. The main soil types are Haplic Calcisols, Calcic Cambisols and Calcic Kastanozems, sequentially as the AI increases (Food and Agriculture Organization, 1993).

Fifty-two sites were selected along the aridity gradient from July to August in 2012, with an interval of approximately 50–100 km. The longitudes range from 91°15′E to 120°21′E, and the latitudes range from 39°51′N to 50°30′N (Fig. 1). At each site, five sub-plots (1 m × 1 m) were selected within one 50 m × 50 m plot (one in the center and one in each corner). The above-ground net primary production (ANPP) was obtained from Wang et al. (2014). Twenty soil cores (0–10 cm depth, 2.5-cm diameter) were collected randomly within each sub-plot and homogenized. Visible roots and stones were removed and fresh soil samples were sieved (< 2 mm) in-situ. A subsample was stored in a plastic bag at 4 °C in the field and immediately frozen at −20 °C after it was returned to the laboratory for biological analysis; a second subsample was air dried and stored in a cloth bag for the measurement of abiotic properties. More detailed descriptions of the test region, climate data, soil type, vegetation type and sampling protocol can be found in Wang et al. (2014) and Feng et al. (2016).

2.2. Soil physicochemical properties and measurement of soil organic N fractions

Values of soil pH, soil organic C (SOC), total N (TN), microbial biomass C (MBC) and microbial biomass N (MBN) were obtained from Wang et al. (2014). Values of soil particle sizes were derived from Wang et al. (2016), and values of soil exchangeable ammonium (NH_4^+) and nitrate (NO_3^-) were obtained from Liu et al. (2017).

Soil organic N fractions were analyzed using an acid-hydrolysis method as described by Stevenson (1996). Briefly, soil samples were heated with 20 mL 6 M HCl in an autoclave at 1 kg cm^{-2} for 6 h. The mixture was cooled, filtered through 30–50 μm sintered discs, and aliquots were taken for the determination of hydrolysable NH_4^+, amino acid-N, amino sugar-N and hydrolysable total-N (Stevenson, 1996). Hydrolysable unknown-N was obtained by subtracting the hydrolysable NH_4^+, amino acid-N and amino sugar-N from the hydrolysable total-N. Soil acid insoluble-N was determined by subtracting the total hydrolysable N from the TN (Stevenson, 1996). For an accurate calculation of the SON fractions, soil inorganic N (exchangeable NH_4^+ and NO_3^-) was subtracted from hydrolysable SON fractions (Bremner, 1965).

2.3. Analyses of soil N-hydrolyzing enzyme activities

Protease activity (EC 3.4.2.21-24) was determined following the method described by Ladd and Butler (1972). Briefly, 1.00 g soil sample was incubated with 5 mL of Tris buffer (0.05 M, pH 8.1) and 5 mL of 2% Na-caseinate solution (W/V) at 50 °C for 2 h. Then, the reaction was terminated by adding 5 mL trichloroacetic acid (0.92 M). The tyrosine concentration was measured using the colorimetrical method at 700 nm after 5 min (Ladd and Butler, 1972). The same procedure was followed for the control, except that the Na-caseinate was added after the incubation. Protease activity was calculated by subtracting control absorbance from sample absorbance, and expressed as μg tyrosine g^{-1} soil h^{-1}.

Soil amidase activity (EC 3.5.1.4) was assayed using method

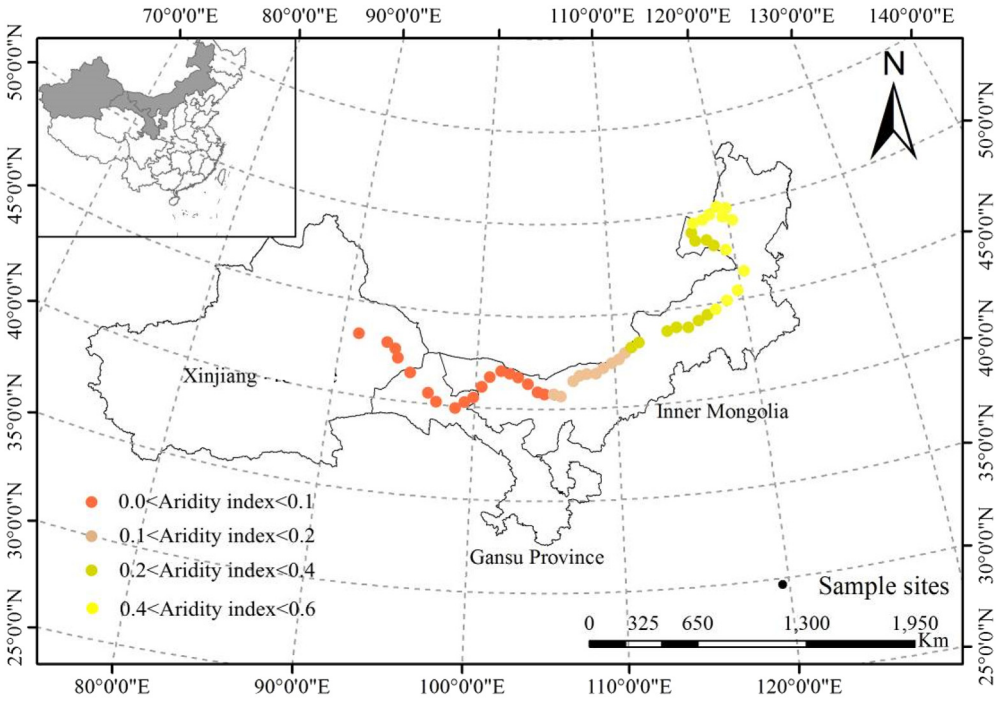

Fig. 1. Geographic distribution of sampling sites. Fifty-two soil samples were collected along a 3700 km transect in arid and semiarid grasslands of northern China. The transect primarily covers an aridity gradient (0.03 < aridity index < 0.57). The aridity index is defined as the ratio of mean annual precipitation to mean annual potential evapotranspiration.

established by Frankenberger and Tabatabai (1980). In brief, 2.00 g soil samples were incubated with 3.6 mL of sodium borate buffer (0.1 M, pH 8.5) and 0.4 mL of 0.5 M formamide at 37 °C for 2 h. Then, 16 mL potassium chloride (0.2 M)-silver sulfate (0.01 M) was added to stop the reaction. The NH_4^+ concentration was measured colorimetrically at 660 nm after 30 min. The same procedure was followed for the control, except that the formamide was added after the incubation. Soil amidase activity was calculated by subtracting control absorbance from sample absorbance, and expressed as $\mu g\ NH_4^+\ g^{-1}\ soil\ h^{-1}$.

Soil *N*-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.14) and soil potential peptidase activities (EC 3.4.11-19, including leucine aminopeptidase, glutamate aminopeptidase and aspartate aminopeptidase) were measured with fluorescent substrates, including 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC) (Marx et al., 2001). Briefly, 2.00 g soil was mixed thoroughly with 200 mL of 1 mmol L^{-1} sodium azide (to prevent microbial activity) using a magnetic stir plate for 15 min. Then, 50 μL of this soil homogenate, 100 μL of 200 μmol L^{-1} substrate solution and 50 μL of 0.2 M acetate buffer (pH 5.0) were added to a 96-well flat-black-bottomed microplate (NUNC, Denmark) and incubated for 3 h at 30 °C. The substrate used for leucine aminopeptidase, glutamate aminopeptidase, aspartate aminopeptidase and NAG was L-leucine-AMC, L-glutamic acid-γ-AMC, aspartic acid-AMC and 4-MUB-*N*-acetyl-β-D-glucosaminide, respectively. After incubation, the reaction was stopped with 50 μL of 1 M NaOH and the fluorescence was read using the multifunctional microplate reader Tecan Infinite 200 PRO (TECAN Group Ltd., Mannedorf, Switzerland). The activities of leucine aminopeptidase, glutamate aminopeptidase and aspartate aminopeptidase were expressed as nmol AMC g^{-1} soil h^{-1}. The activity of NAG was expressed as nmol MUB g^{-1} soil h^{-1}. The potential peptidase activity was the sum of the leucine aminopeptidase, glutamate aminopeptidase and aspartate aminopeptidase.

2.4. Statistical analysis

Regression analyses were performed using Origin 9.0 software (OriginLab Corp., Northampton, MA, USA). Ordinary least squares (OLS) linear fitting or polynomial fitting methods was used to analyze the relationship of AI with the concentrations and proportions (TN) of soil SON fractions and soil N-hydrolyzing enzyme activities. Pearson correlation coefficients were calculated for the relationships of climatic (AI), biotic (ANPP, MBC and MBN) and abiotic factors (soil pH, soil texture, SOC, TN and organic P) with soil N-hydrolyzing enzymes (SPSS 18.0 software, Chicago, IL, USA).

Redundancy analysis (RDA) was conducted to compare the relative contributions of climatic, biotic and abiotic factors on the proportion of soil N fractions to TN using Canoco Software (Version 4.5, Microcomputer Power, Ithaca, New York). Environmental factors that had high variance inflation factor (VIF > 20) were removed from the model to eliminate multicollinearity. The statistical significance of the factors of variance was tested using a Monte Carlo permutation test (Supplement Table S3; Ter Braak and Smilauer, 2002).

Structural equation modeling (SEM) was used to evaluate the direct and indirect effects of climatic (AI), biotic (ANPP and microbial activity [MB]) and abiotic factors (soil pH, soil clay content and SON fractions) on soil N-hydrolyzing enzymes. Because the nonlinear relationships of AI with SON fraction concentrations and soil N-hydrolyzing enzymes (protease, peptidase and NAG), SEM were conducted independently for arid and semiarid sites to ensure the linearity in the relationships between different variables. A prior model was established based on the known relationships among the drivers of soil N-hydrolyzing enzymes (Supplement Fig. S2). The concentrations of five SON fractions were highly correlated (Pearson's r ranges from 0.808 to 0.968, $P < 0.001$; Supplement Table S1). Thus, principal components analysis (PCA) was conducted to reduce the number of variables. The first component of SON fractions from PCA (SONC) explained 86% of the total variance of SON fractions and was highly related to all SON fraction concentrations (Pearson's r ranges from 0.877 to 0.977, $P < 0.001$; Supplement Table S1). Similarly, the first component of MBC and MBN was also extracted as MB (explaining 98% of the total variance), because MBC and MBN were highly correlated (Pearson's $r = 0.954$, $P < 0.001$). After PCA analyses, both SONC and MB were introduced as new variables into the model. The distributions of all endogenous variables were examined, and their normalities were tested. Soil clay content was square-root transformed, and ANPP and soil enzyme activities were $\log_e$-transformed to improve normality. The models were then parameterized using data from arid and semiarid sites, respectively. The chi-squared test (χ^2; the model has a good fit when χ^2 was low and the *P*-

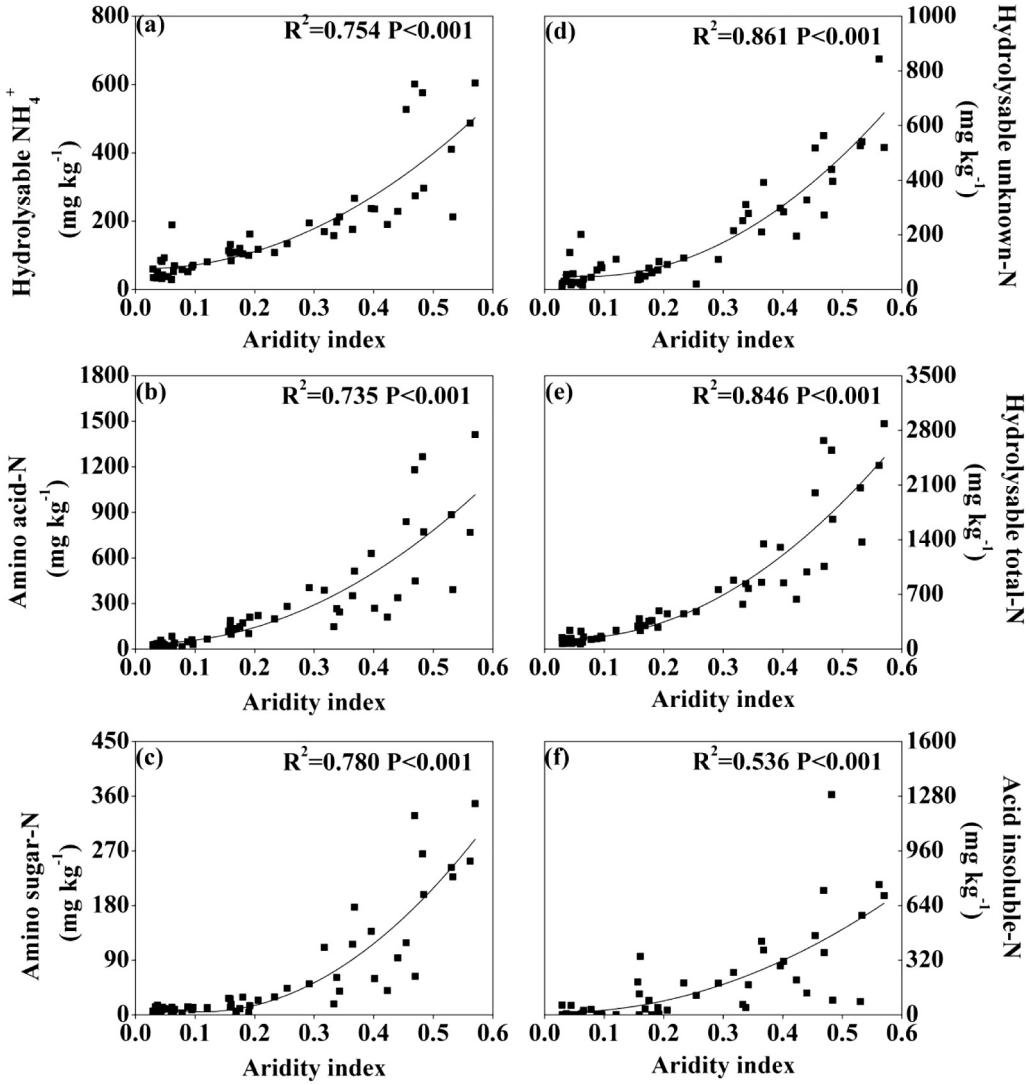

Fig. 2. Concentrations of soil organic N fractions along the aridity gradient. Lines are obtained by a second-order polynomial fitting based on 52 sample sites. Aridity index defined as mean annual precipitation/potential evapotranspiration.

value > 0.05) and the root-mean-square error of approximation (RMSEA; the model has a good fit when 0 ≤ RMSEA ≤ 0.05) were used to test the overall goodness of fits for SEM (Mulaik et al., 1989). Given that some variables were not normally distributed, the fitness of the model was further confirmed using the Bollen-Stine bootstrap test (the model has a good fit when 0.10 < bootstrap $P \leq 1.00$). Our prior models in both arid and semiarid sites gave satisfactory fits to our data, as suggested by these different goodness-of-fit metrics (Fig. 6). All the SEM analyses were conducted by Amos 18.0 (IBM, SPSS, New York, USA).

3. Results

3.1. Soil organic nitrogen fractions in relation to climatic, edaphic and biotic factors

The relationships between concentrations of SON fractions and AI were non-linear (second-order polynomials) across the aridity gradient. In drier sites with low AI (AI < 0.2), the increasing slopes were shallow, but all SON fractions increased significantly as AI increased into semiarid sites (AI > 0.2; Fig. 2). In both arid and semiarid sites, the concentrations of SON fractions were generally positively correlated with SOC, TN, soil silt- and clay-sized particles and biotic factors (MBC, MBN and ANPP) ($P < 0.05$; Supplement Table S2).

The proportion of hydrolysable NH_4^+ to TN declined linearly as AI increased ($P < 0.01$, Fig. 3, Supplement Fig. S1). Conversely, proportions of amino acid-N, amino sugar-N and acid insoluble-N increased linearly as AI increased ($P < 0.01$, Fig. 3, Supplement Fig. S1). Inorganic N accounted for only approximately 6% of TN on average, and showed an overall declining trend as AI increased in arid sites (Fig. 3). The largest soil N fraction shifted from hydrolysable NH_4^+ (approximately 40% in the driest regions) to amino acid-N (approximately 50% in the wettest part) as AI increased, having equal proportions (about 31% of TN) at AI = 0.2.

Results of RDA showed that SOC and soil texture were the most important factors influencing SON composition, explaining 26% and 15% of the overall variations in SON composition, respectively (Supplement Table S3). The proportion of hydrolysable NH_4^+ (labile SON fraction) to TN was the most negatively correlated with SOC, while amino acid-N/TN and amino sugar-N/TN (intermediate bioavailable SON fractions) had strong positive relationships with SOC and soil silt content (Fig. 4). The proportion of acid insoluble-N (the most stable SON fraction) was the most positively correlated with soil clay content (Fig. 4).

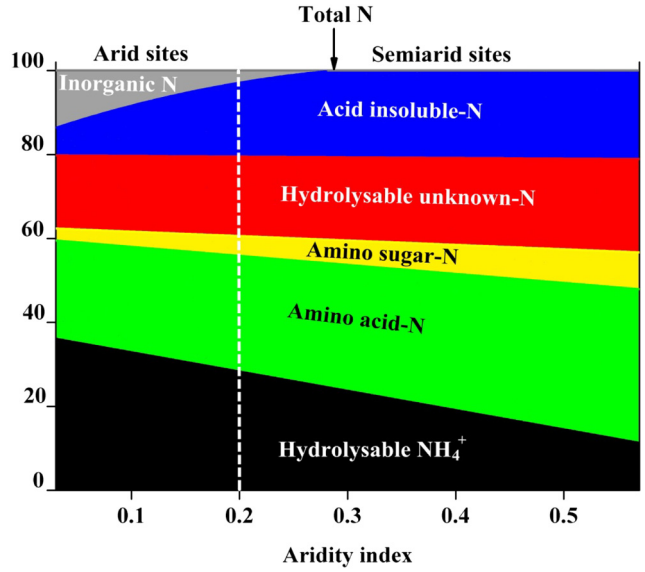

Fig. 3. The proportions (to total N) of soil N fractions along the aridity gradient. Lines are obtained by linear or polynomial fitting based on 52 sample sites. The area between two solid lines represents the proportion of soil N fraction to total N. The dashed line (AI = 0.2) represents the boundary between arid and semiarid regions according to the definition of United Nations Environment Programme (1992). At AI = 0.2, hydrolysable NH_4^+ and amino acid-N have equal proportions (31% of TN).

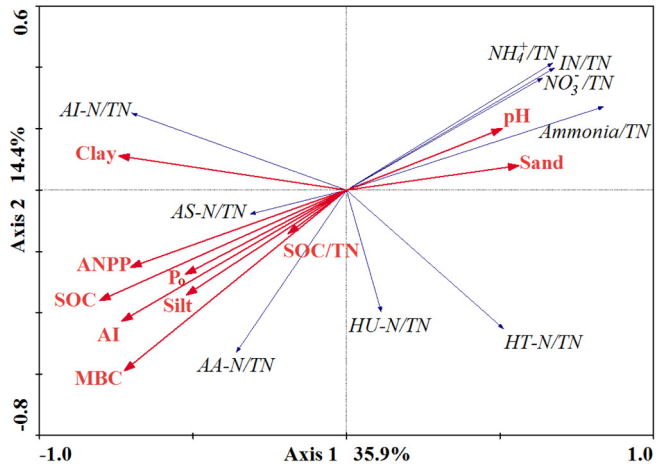

Fig. 4. Soil N composition and environmental variables biplot of redundancy analysis (RDA). AI, aridity index; SOC, soil organic carbon; TN, total N; P_o, organic P; MBC, microbial biomass C; ANPP, above-ground net primary production. Ammonia, hydrolysable NH_4^+; AA-N, amino acid N; AS-N, amino sugar N; HU-N, hydrolysable unknown N; HT-N, hydrolysable total N; AI-N, acid insoluble N; NH_4^+, exchangeable NH_4^+; IN, sum of exchangeable NH_4^+ and NO_3^-.

3.2. Relative contributions of climatic, edaphic and biotic factors on soil N-hydrolyzing enzyme activities

All soil N-hydrolyzing enzyme activities increased as AI increased (Fig. 5). However, soil protease, potential peptidase and NAG activities showed non-linear (second-order polynomial) relationships with AI, with these activities showing little increases as AI increased in arid sites, but increasing significantly in semiarid sites (Table 1; Fig. 5). In contrast, soil amidase activities increased linearly as AI increased across the entire aridity gradient ($R^2 = 0.796$, $P < 0.001$; Fig. 5c).

In arid sites, the relationships of soil protease, potential peptidase and NAG with soil biotic (MBC, MBN, ANPP) and abiotic factors (soil pH, soil texture and soil nutrient concentrations) were generally insignificant, except for the significant negative correlation between NAG and soil pH (Table 1). In contrast, soil amidase activity was positively correlated with SOC, TN, soil biotic factors and soil clay content, but negatively correlated with soil pH and soil sand content (Table 1). However, in semiarid sites, all N-hydrolyzing enzymes (including soil protease, potential peptidase, amidase and NAG) showed positive correlations with SOC, TN and soil biotic factors (Table 1).

In arid sites, our SEM model explained ~60% of the variance for soil amidase activity, and was confirmed by the different goodness of fit parameters used (Fig. 6). The first PCA component of SON fractions (SONC) had a direct positive effect (path coefficient = 0.906) on soil amidase activities (Fig. 6a). The model was also applicable when SOC was incorporated into the PCA component of SON fractions, namely soil organic matter component (SOMC), which had a direct positive effect on soil amidase with a path coefficient of 0.814 (Supplement Fig. S4). Aridity index showed an indirect positive effect on soil amidase activity via SONC (Fig. 6a). In semiarid sites, our SEM model explained 78.4%, 59.8%, 68.6% and 93.8% variations of soil protease, peptidase, amidase and NAG, respectively (Fig. 6b). Microbial biomass had significant direct positive effects on all soil N-hydrolyzing enzymes. AI had direct positive effects on soil peptidase and NAG, while soil pH had direct negative effects on soil protease and NAG.

4. Discussion

Plant and microbial residues are the dominant sources of organic inputs into soil (Kogel-Knabner, 2002), so the distinct increase in the concentrations of all SON fractions as AI increased can be related to the enhancements of ANPP and MBC with increasing AI (Fig. 2; Wang et al., 2014). Despite this trend in SON concentrations, the relative abundance of SON fractions shifted significantly as AI increased (Fig. 3), perhaps responding to the decoupling of plant and microbial responses to small rainfall events in extremely arid sites (Kogel-Knabner, 2002; Schwinning and Sala, 2004). Soil microbes could be activated by smaller rainfall events compared with plants (Schwinning and Sala, 2004), in which case the proportion of organic material originating from microorganisms would be greater in extremely arid sites than in more mesic sites (Xu et al., 2013).

Compared to plant residues, microbial residues commonly have narrower C:N ratio and represent an important source of labile N or easily degradable N (Carrera et al., 2009). The proportion of hydrolysable NH_4^+, which is predominantly derived from the hydrolysis of labile amides (Bremner, 1965), declined as AI increased. In contrast, plant residues commonly have wider C:N ratios and are composed of complex organic polymers, mainly polysaccharides, lignin and phenols (Carrera et al., 2009; Kogel-Knabner, 2002). These C-containing organic polymers can directly adsorb or chemically bind with N-containing compounds (e.g., proteins, peptides, amino sugars etc.), forming complex organic mixtures such as the widely observed lignin-protein complexes (Greenfield, 2001; Stevenson, 1982). This adsorption or binding of N-containing compounds could reduce SON decomposition by microorganisms (Kleber et al., 2007), which perhaps explains why in our study the proportion of amino acid-N and amino sugar-N to TN significantly increased along with the increasing SOC and SOC:TN ratios (Figs. 3, 4), given that amino acid-N was mostly derived from the hydrolysis of protein-peptide N during acid-hydrolysis process (Stevenson, 1982).

In addition to changes in organic residues inputs, variation in SON composition across the aridity gradient could also be related to changes in soil texture (Fig. 4; Supplement Table S3; Bremner, 1965), given that sand, silt, and clay have unique capacities to sorb and stabilize the various SON fractions (Baldock and Skjemstad, 2000; Bremner, 1965; Nannipieri and Eldor, 2009). Organic matter preserved on silt particles is better protected against biological decomposition than organic matter associated with sand particles, but is less protected than organic

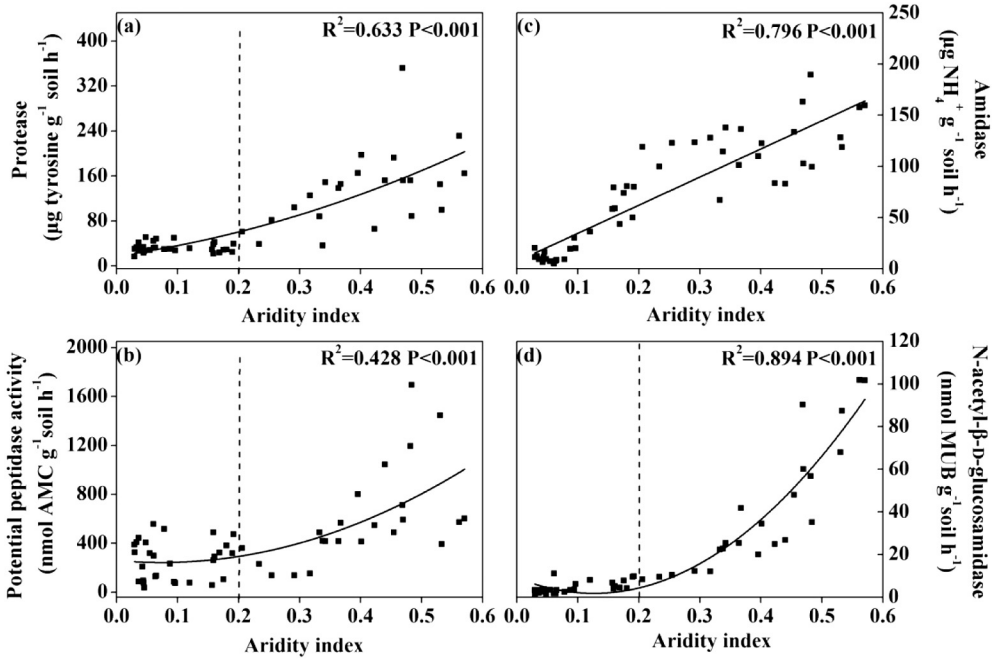

Fig. 5. Variations in N-hydrolyzing enzyme activities along the aridity gradient. The dashed line (AI = 0.2) represents the boundary between arid and semiarid regions. MUB, 4-methylumbelliferone; AMC, 7-amino-4-methylcoumarin.

Table 1
Relationships of climatic, biotic and abiotic factors with soil N-hydrolyzing enzymes.

		Protease	Peptidase	Amidase	NAG
Arid sites	AI	0.139	0.162	0.913**	0.374
	pH	−0.072	−0.302	−0.379*	−0.414*
	Sand	0.139	−0.116	−0.616**	−0.258
	Silt	0.014	0.270	0.364	0.208
	Clay	−0.165	−0.063	0.590**	0.359
	SOC	0.263	0.109	0.743**	0.400
	TN	0.060	0.273	0.676**	0.365
	P_o	0.159	−0.366	−0.059	−0.099
	MBC	0.296	0.234	0.701**	0.339
	MBN	0.211	0.197	0.637**	0.329
	ANPP	0.249	−0.016	0.675**	0.370
Semiarid sites	AI	0.493*	0.681**	0.419*	0.914**
	pH	−0.282	−0.644**	−0.185	−0.750**
	Sand	−0.488*	−0.358	−0.700**	−0.589**
	Silt	0.541*	0.330	0.690**	0.616**
	Clay	0.483*	0.225	0.678**	0.413*
	SOC	0.535*	0.634**	0.616**	0.683**
	TN	0.629**	0.531*	0.703**	0.632**
	P_o	0.325	0.431*	0.587**	0.863**
	MBC	0.621**	0.574**	0.633**	0.775**
	MBN	0.591**	0.581**	0.625**	0.658**
	ANPP	0.623**	0.105*	0.446**	0.557**

SOC, soil organic C; TN, total N; P_o, soil organic P; MBC, microbial biomass C; MBN, microbial biomass N; ANPP, above-ground net primary production; NAG, *N*-acetyl-β-D-glucosaminidase.

* $P < 0.05$ based on the Pearson correlation coefficients.

** $P < 0.01$ based on the Pearson correlation coefficients.

matter associated with clays (Hassink, 1997; Roscoe et al., 2000). Therefore, the proportions of intermediate bioavailable N pools (i.e., amino acid-N and amino sugar-N) are most positively correlated with silt particles (Fig. 4). However, the proportion of acid insoluble-N, which is mainly composed of heterocyclic compounds (Stevenson, 1982) and is the most stable N pool (Lü et al., 2013), is regulated primarily by clay particles (Fig. 4).

The overall reduced proportion of hydrolysable NH_4^+ to TN with increasing AI, as well as the increased proportion of amino acid-N, amino sugar-N and acid insoluble-N, suggest that soil N was gradually transformed into more stable SON pool with increasing AI. These changes in SON composition along the aridity gradient could help to evaluate how climatic factors affect N storage and supply at regional scale in different climate zones (Post et al., 1985; Tian et al., 2006). In extremely arid sites, the higher proportion of labile SON fractions might promote rapid biological N supply in response to rainfall events (Austin et al., 2004). However, the lack of more stable SON fractions may have a negative influence on SON storage as well as the sustainability of soil structure and resistance to wind erosion (Reynolds et al., 2007), making arid ecosystems more vulnerable to future climate change.

Most of the organic N polymers must be first mineralized to mineral NH_4^+ by depolymerases before taken up by plants and soil microorganisms (Geisseler et al., 2010). In soils with an array of substrates, the synthesis of enzymes that target relatively abundant substrates may be energetically favorable for microbial decomposers (German et al., 2011; Lehmann et al., 2008). Therefore, in arid sites where hydrolysable NH_4^+ (mostly derived from labile amides by acid-hydrolysis method; Stevenson, 1982) was relatively abundant (Fig. 3), soil amidase activity was higher than other enzymes and increased significantly with increasing AI (Fig. 5). In contrast, soil protease, peptidase and NAG activities were generally low, due to the low concentration and proportion of amino acid-N (mostly derived from protein-peptides by acid-hydrolysis method; Stevenson, 1982) and amino sugar-N (Figs. 2, 3). These results are consistent with those of German et al. (2011), who found that α-glucosidase activity was induced only when the proportion of starch to organic matter was > 10%. In arid sites in our study, the low activities of protease, peptidase and NAG suggest that SON turnover through hydrolysis of relatively complex SON fractions was restricted. Instead, the hydrolysis of more labile SON via amidase activity may play more important roles in SON turnover (Fig. 5). In extremely arid sites, the allocation to N-hydrolyzing enzymes in relation to the relative abundance of SON fractions may be particularly important, as arid soils are usually limited by both energy (C) and resources (Delgado-Baquerizo et al., 2013). The preferential use of labile SON by amidase has implications for understanding SON turnover in extremely arid sites characterized by low precipitation and pulsed rainfall events (Austin et al., 2004), especially as it relates to higher aridity coupled

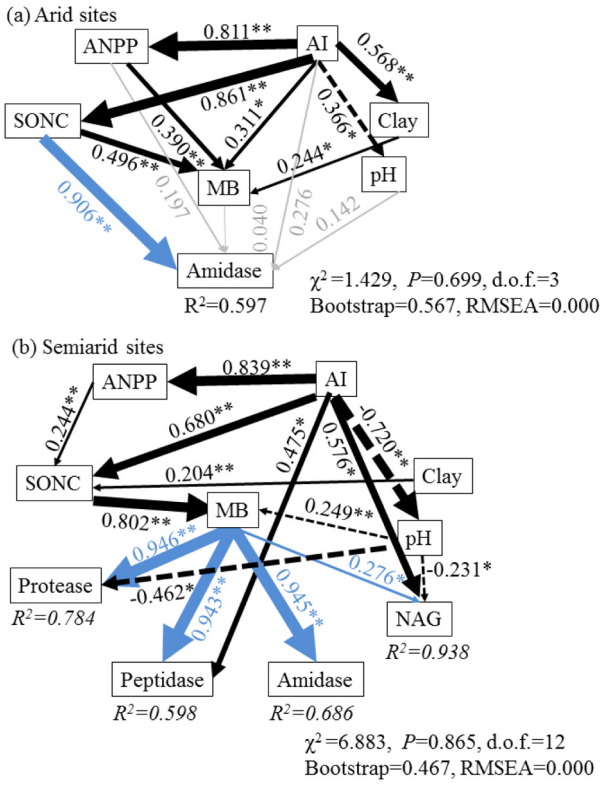

Fig. 6. Structural equation model assessing the direct and indirect effects of multiple drivers of climatic, edaphic and biotic factors on soil N-hydrolyzing enzyme activities in arid and semiarid sites. Numbers adjacent to arrows indicate the effect size of the relationship; arrow thickness is proportional to the magnitude of path coefficient. Continuous and dashed arrows indicate positive and negative relationships, respectively. R^2 denotes the proportion of variance explained by the model. Significance levels are: $^*P < 0.05$; $^{**}P < 0.01$. AI, aridity index; ANPP, above-ground net primary production; SONC, soil organic N component, the first component of soil organic N fractions (hydrolysable NH_4^+, amino acid-N, amino sugar-N, hydrolysable unknown-N and acid insoluble-N) from principal components analysis; MB, microbial biomass, the first component of microbial biomass C and microbial biomass N; NAG, *N*-acetyl-β-D-glucosaminidase.

with more extreme rainfall events in arid sites under global climate change (Feng and Fu, 2013).

It is well established that N-hydrolyzing enzymes in soils originate primarily from microorganisms (Kandeler et al., 2011; Tabatabai and Dick, 2002). Although amidase activity was positively correlated with MBC and MBN (Table 1), our SEM model indicated that the direct regulation of amidase by soil microorganisms was insignificant in arid sites (Fig. 6). In fact, the strongest direct effect on soil amidase production in arid sites was the first component of SON fractions from the PCA (Fig. 6). The model was also applicable when SOC was incorporated into the PCA component, namely the soil organic matter component (SOMC) (Supplement Fig. S4). In arid sites that usually have low concentrations of both C and nutrients (Delgado-Baquerizo et al., 2013; Wang et al., 2014), soil enzyme production may be more limited by soil energy and nutrient availability. This is consistent with previously observed repression of protease production in sandy soils where activity is limited by N and sulfur (Sims and Wander 2002). Similarly, Ladwig et al. (2015) also found significantly lower enzyme activities in interspace soils between two canopies than under canopies in desert ecosystems. Since water films are commonly thin and soil pores may be disconnected in arid sites (Austin et al., 2004), the slow response of amidase to soil microorganisms may also be related to the restricted diffusion and separation of extracellular enzymes from microorganisms (Schjønning et al., 2003). Moreover, the adsorption of extracellular enzymes on organic materials or soil colloids may also contribute to the disconnection between microorganisms and soil amidase activity in arid sites (Burns, 1982).

In arid sites, the insignificant direct effect of microorganisms on soil amidase activity (Fig. 6), coupled with the overall low and lack of changes in soil protease, peptidase and NAG activity (Fig. 5), indicates that the microbial influence on SON turnover via enzymatic mineralization is weak. In contrast, in semiarid sites, all soil N-hydrolyzing enzymes significantly increased with increasing AI (Fig. 5) and showed significant direct responses to soil microorganisms in our SEM model (Fig. 6), suggesting strong microbial regulation of SON turnover via enzymatic mineralization (Geisseler et al., 2010). Our results therefore indicate that the pathways of microbial control of SON turnover are different in arid and semiarid sites.

Besides enzymatic mineralization, the direct uptake of small organic N molecules has been identified as an essential alternative pathway of SON turnover for both plants and microorganisms (Geisseler et al., 2009; Näsholm et al., 2009; Yang et al., 2016). In extremely arid sites where SOC and soil C:N are low (Wang et al., 2014) and a greater proportion of N is derived from microorganisms compared to more mesic sites (Xu et al., 2013), direct organic N uptake might be favored, including as C source (Geisseler et al., 2010). In addition, high atmospheric N inputs, small losses of inorganic N through leaching (Fig. 3; Liu et al., 2017; Wang et al., 2014), and decomposition of organic matter by solar radiation (Austin and Vivanco, 2006; Foereid et al., 2010), could all promote the accumulation of inorganic N in arid soils. These abiotic processes could also provide an alternative pathway for soil N supply to plants and microorganisms, inhibiting enzymatic mineralization of SON (Allison et al., 2011; Geisseler et al., 2010). The dominant control of abiotic processes relative to biotic processes on soil nutrient cycles has been confirmed by previous studies in these arid sites, including soil N (Wang et al., 2014), phosphorus (Feng et al., 2016) and sulfur cycling (Luo et al., 2016a). In addition, Luo et al. (2016b) observed decoupled biotic linkages of elements between soils and plants in extremely arid sites. Therefore, the low activities of SON mineralization via enzymes in arid sites in this study correspond well with previous studies that indicated weak biotic control of soil nutrient cycling in extremely arid sites. Further work is required to determine patterns of direct organic N assimilation and abiotic controls on soil N cycling, which will help further explain mechanisms of ecosystem N cycling in drylands.

5. Conclusions

Our results provide evidence that the composition of SON and its turnover via enzymes differ between arid and semiarid sites. The largest SON fraction in arid sites was hydrolysable NH_4^+, but in semiarid sites it was amino acid-N. The activities of enzymes that hydrolyze relatively complex SON fractions (including protease, peptidase, and NAG) were negligible, and showed little response to changes in AI in arid sites. In contrast, all these enzymes increased significantly with increasing AI in semiarid sites. Remarkably, our SEM analyses showed that the direct effect of soil microbial activities on soil amidase was insignificant in arid sites, suggesting that microbial control of SON turnover via enzymatic mineralization was repressed. However, soil microbial activity in semiarid sites had significant direct effects on all soil N-hydrolyzing enzymes (i.e., protease, peptidase, amidase and NAG), suggesting strong microbial regulation of SON turnover through enzymatic mineralization. By providing evidence for the different patterns of SON turnover through enzymatic mineralization between arid and semiarid sites, our results advance our understanding of the extent of microbial control (Sinsabaugh et al., 2015) and biotic versus abiotic control of soil N cycling in drylands (Wang et al., 2014).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.04.020.

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